Exhibit 1 - Report of First Clinical Trial

Acambis

CLINICAL STUDY REPORT

Phase 1, Dose Escalation, Safety and Immunogenicity of Two New Attenuated Vaccine Strains for Enterotoxigenic *E. coli* in volunteers

Inpatient study

(Protocol# VTU983)

BB-IND#: 7922

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Study Start Date:		Study Completion Date:		Date of Report:	July 02, 2001

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SYNOPSIS

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Investigational product: Enterotoxigenic *E. coli* (ETEC) vaccine PTL-ETEC-002 and PTL-ETEC-003		
Active ingredient: Live fresh washed ETEC bacteria	**Page:**	

Title: Phase 1, Dose Escalation, Safety and Immunogenicity of Two New Attenuated Vaccine Strains for Enterotoxigenic *E. coli* in volunteers

Investigators: David Sack, MD (PI) and (any subinvestigators?)

Study centre: General Clinical Research Center at Johns Hopkins Hospital, Baltimore, MD

Analytical site: Clinical Laboratory - Johns Hopkins Hospital and Research Microbiology - Department of International Health in the Johns Hopkins University School of Hygiene

Study Period of clinical phase: **Date first patient admitted for enrollment (vaccination):** 26 October 1998 **Date first patient enrolled (vaccinated):** 27 October 1998 **Date last patient completed:** 09 March 1999 (14 days post last vaccination)	**Clinical Phase**: I

Objectives:

- To recruit volunteers according to the protocol's inclusion and exclusion criteria, to admit these volunteers to the General Clinical Research Center (GCRC) and give them a dose of vaccine candidate, to monitor them clinically and manage any symptoms which might occur.
- To monitor the fecal excretion of the vaccine candidate strains.
- To measure the serological response as determined by the antibody titers in the serum and the supernate of lymphocytes cultured *in vitro*.

Methodology: Single-center, open-label, inpatient, safety and immunogenicity study to evaluate two new attenuated strains of enterotoxigenic *E. coli* vaccine (PTL-ETEC-002 and PTL-ETEC-003). Vaccine was administered orally as a single dose on Day 0 to eligible inpatient volunteers. Both vaccine strains were given in a dose-escalation design with escalation to the higher dose level dependent on lack of clinically significant effects at the lower dose level, the planned escalation was from 5×10^7 bacteria to 5×10^9 bacteria to 5×10^{10} bacteria.

Number of subjects (*planned and analysed*): 30 planned (15 for each vaccine), 3 volunteers for each strain (PTL-ETEC-002 and PTL-ETEC-003) to receive 5×10^7 bacteria, 6 volunteers for each strain (PTL-ETEC-002 and PTL-ETEC-003) to receive 5×10^9 bacteria, and 6 volunteers for each strain (PTL-ETEC-002 and PTL-ETEC-003) to receive 5×10^{10} bacteria. A total of 27 volunteers in six groups received vaccine, a total of 6 in 5×10^7 bacteria group, a total of 11 in 5×10^9 bacteria group, and 10 in 5×10^8 bacteria group.

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Diagnosis and main criteria for inclusion: Healthy, non-immunocompromised, male or female inpatient volunteers, >18 or <50 years of age with none of the following: clinically significant medical history, physical examination, or screening laboratory examinations (complete blood count with differential, blood chemistry, urinalysis), negative serologies for HbsAg, HCV, HIV, negative urine HCG within 4 days of immunization (women only), and volunteers over the age of 40 with a normal EKG. Volunteers were required to complete a training session, provide written informed consent, and demonstrate comprehension of the protocol procedures and knowledge of diarrhea, ETEC bacteria by passing a written examination. Volunteers were excluded from the study if they had a chronic illness, regular use of laxatives or abnormal stool pattern, if they travelled to a developing country within 5 years, if they previously participated in an ETEC study, or if antibiotics were used within 7 days of vaccination.

Test product, dose and mode of administration, batch number: Sequential groups of volunteers were to receive oral doses of $5x10^7$, $5x10^9$ and $5x10^{10}$ CFU, batch numbers????, administered by mouth, 120 ml of buffer (sodium bicarbonate solution [1.33% in water]), then 1 minute after buffering of stomach contents, 30ml of the same sodium bicarbonate solution (filter sterilized) containing the vaccine.

Reference product: none

Duration of study drug treatment: Single oral administration of one of 3 dose levels of vaccine.

Criteria for Evaluation:

Safety:

Reactogenicity was ascertained by analysing documented signs and symptoms of illness where the hospitalized subject was monitored twice daily on the day of immunization and for the 6 days after immunization. Vital signs including heart rate, blood pressure, respiratory rate and temperature, were performed three times a day while hospitalized. Also documented, was the date, grade, and the weight of all stools passed during this hopitalization period; the first two stools were collected daily and sampled for microbiological examination and assessment of bacterial shedding and tested for occult blood. Fluid intake and output were measured during this hopitalization period. If no symptoms developed following vaccination, on Day 4 subjects were given Ciprofloxacin 500 mg for three days. If subjects developed symptoms prior to Day 4, antibiotic treatment could be initiated at the Investigator's discretion. Subjects were to be discharged on Day 6. After hospital discharge, subjects were asked to contact the Vaccine Testing Unit in the event of late symptoms. The subjects were asked to return to the outpatient clinic on days 10 and 14 for an interval history.

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Immunogenicity:
The immunogenicity of the vaccine was evaluated by antibody response to vaccine strains and to IgG and IgA antibody to CFA II. Blood samples for serology (serum and lymphocyte specimens) were to be collected prior to vaccination, on Day 9 and Day 14 after vaccination according to schematic and protocol but samples were obtained prior to vaccination, and on Days 7, 10 and 14.

Statistical methods: This sample size did not allow for statistical methodology. Adverse events were summarized by frequency of occurrence, number of subjects experiencing adverse events, severity and relationship to investigational vaccine. Immune response to the vaccine was determined qualitatively without pre-study definitions of positive and negative responders.

Safety results:

No serious vaccine related adverse events were reported. No clinically significant trends in adverse events, vital signs or screening clinical laboratory test were observed in regard to subject safety.

Six (6) volunteers received 5 x 10^7 per dose of strain PTL-ETEC-002 (N=3) and PTL-ETEC-003 (N=3). No significant adverse events were seen and the study proceeded to the next highest dose group 5 x 10^9. Eleven (11) volunteers received 5 x 10^9 (N=5 for strain PTL-ETEC-002 and N=6 for strain PTL-ETEC-003) and adverse events including moderate gas/cramps (N=3), one episode of vomiting, and two cases of grade 3 diarrhea were seen at this dose group. Therefore the next dose group received a reduced dose, 5 x 10^8 per dose strain. Ten (10) volunteers received 5 x 10^8 (N=6 for strain PTL-ETEC-002 and N=4 for strain PTL-ETEC-003) and two cases of moderate gas/cramps and one episode of vomiting were noted. **Table 1** delineates the incidence of symptoms per dose group. None of the volunteers developed an elevated temperature. In neither case of diarrhea, vomiting or gas/cramps did the volunteers require restricting or changing activities.

Efficacy results:

Excretion of vaccine strains: Among those study subjects receiving a dose of 5 x 10^7 CFU, the vaccine was recovered from the stools of all of 6 volunteers at some time. It was recovered the same day as vaccination from two volunteers and continued to be excreted for up to four days in two volunteers. Of those who received the 5 x 10^8-dose level, 9 of 10 volunteers excreted the vaccine strain at some time, but one volunteer never excreted the strain. Again, some volunteers continued to excrete for up to four days. Of those who received a dose of 5 x 10^9, all of 11 volunteers excreted the vaccine strain and all continued to excrete for four days, compared to only 4 of 16 who received lower doses who continued to excrete for four days ($p<0.0001$, Fisher's Exact Test). There was no difference in the frequency or duration of the

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excretion of the two vaccine strains when given at comparable doses.

Serology results:

Immune reponse to the vaccine was assessed by determining serum antibody levels at various times (7, 10 and 14 days) following vaccination compared to baseline values. Immune repsonse was also assessed by a modified antibody secreting cell assay (ALS, antibody lymphocyte supernatant assay) in which peripheral blood monocytes sampled 7 and 10 days following immunization were cultured and their supernant fluids assayed by ELISA for antigen specific antibodies. The titers of serum IgG and IgA anti-CFA did not change significantly between the sample collected prior to vaccination and those collected after vaccination. It was noted that there was great variability between the titers from one volunteer to others. Titers of anti-IgG and IgA from the ALS specimens increased significantly between the preimmune specimen collected on Day 0 and the specimen collected on Day 7 after vaccination. By Day 10-post vaccination, the titers decreased.

Conclusion: The vaccine strains were associated with mild and moderate symptoms by protocol and/or case report form definition. Neither IgG nor IgA serum anti-CFA I antibody responses were detected in any of the volunteers. Anti-CFA responses were seen in the specimen from the ALS specimen, which peaked on Day 7-post vaccination and returned to near baseline by Day 10-post vaccination.

These data suggest that it is safe and form a basis for further evaluation of PTL-ETEC-002 and PTL-ETEC-003 with outpatient study. The outpatient study should include an assessment of duration of excretion and a control group to better assess the relation of symptoms with the vaccines. The lymphocyte antibody response should also be continued in the outpatient study, as it appeared to a more sensitve assay for immune response than serum antibodies.

EPORT SIGNATURES

ur signature(s) below confirm the accuracy and content of the data contained within this report and our respectiv nalyses and summaries thereof:

nvestigator:
. Louis Bourgeois, PhD

_____ _____
Signature date

edical Monitor:

_____ _____
Signature date

uthors of Report:
aria Berkheimer, The Total Approach, Inc.

_____ _____
Signature date

ynthia K. Lee, Ph. D., Acambis Inc.

_____ _____
Signature date

ETHICS

2.1 Institutional Review Board (IRB)

Prior to implementation, the study protocol was approved in writing, by the IRB of the Johns Hopkin University School of Medicine or Johns Hopkins Hospital. All subject-related procedures were carried out at General Clinical Research Center (GCRC) at Johns Hopkins Hospital, Baltimore, MD

IRB membership was maintained according to federal guidelines set forth in CFR Part 56. Details o the constitution of the IRB, including the names of their Chairs, are held on file at the Vaccine Testing Unit (VTU), The Johns Hopkins University School of Medicine. For a copy of the IRB approva details refer to **Appendix 12.1**.

This study was conducted under an IND (BB-IND#7922, Serial #001) held by David A. Sack, M.D and later transferred investigator responsibilities to A. Louis Bourgeois, M.D. The Johns Hopkin University. The study was financed by Acambis, Inc. previously known as Peptide Therapeutics.

2.2 Ethical Conduct of the Study

The study was carried out in accordance with the ethical principles outlined in the Declaration o Helsinki, 1964 and subsequent amendments[1].

2.3 Subject Information and Consent

In response to advertisements published in local papers? (see example in **Appendix 12.4**), subject interested in participation contacted the VTU and were invited to attend a briefing at the VTU at which the study was outlined. Written information/consent forms were given to the subject to study. Fo participants at VTU, there were separate consent forms for the collection of screening blood sample and for study participation. Examples of all consent forms are given in **Appendix 12.2**. Subject interested in participation were invited back to VTU for the collection of screening samples Witnessed written informed consent was obtained by study personnel, prior to any study-related procedure. Enrollment to the trial took place a few days prior to, or on the day of the first vaccination For eligible subjects, enrollment comprised a final briefing by one of the study personnel, and written comprehension test (in which subjects were required to score at least 70%).

INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

The following key personnel from the VTU were involved in the management of the inpatient study and th subjects enrolled:

Medical Monitor: David Sack, MD

Principal Investigator: David Sack, MD

Sub-investigator:

Sub-investigator (immunology):

Sub-investigator (bacteriology):

Statistician: None

Study Coordinator:

Study Nurse:

Curricula vitae for key personnel are located in **Appendix 12.2**. A signature list for the key site personnel i presented in Appendix?.

The Study Drug was administered by [insert name] and was independent of any of the clinical or serologica evaluations in the study.

Johns Hopkins Hospital Laboratory, 600 Wolfe Street, Baltimore, MD 21205 performed clinical chemistry and routine hematology assays.

The following personnel were responsible for the bacteriological and immunological assays:
[insert names].

Management of the clinical data (i.e. all data with the exception of those derived from the bacteriological and immunological assays) was carried out by Vaccine Testing Unit. [insert names]

INTRODUCTION

Enterotoxigenic *Escherichia coli* (ETEC) is the major etiological agent associated with traveller's diarrhea in many parts of the developing world and is a major cause of morbidity in both military and civilian travellers to these regions. It also causes up to 380,000 deaths in infants and young children in endemic regions.

There is currently no licensed vaccine available for the prevention of ETEC disease, although there is candidate vaccine being developed by SmithKline Beecham currently undergoing phase III evaluation. Thi consists of an inactivated whole cell preparation of five different ETEC strains, combined with recombinan cholera toxin B subunit (CT-B), which is administered as two oral doses.

The vaccines being tested in this study consists of live attenuated strains of ETEC for oral delivery. Simila live attenuated bacterial vaccines have been developed against *Salmonella typhi* and *Vibrio cholera*. Liv attenuated ETEC organisms colonise the intestinal mucosa of vaccinees, providing prolonged exposure to antigen, and will avoid the need for the addition of exogenous adjuvant. It is hoped that a single dose o vaccine will prove to be effective.

ETEC pathogenicity is well understood; fimbrial Colonisation Factors mediate adherence to the surface of th intestinal epithelium where the bacteria secrete enterotoxins, which are responsible for the debilitating watery diarrhea. Protective immunity requires both a secretory IgA response against the Colonisation Factors to block adherence and toxin neutralising antibodies.

A spontaneous toxin deletion mutant of a diarrheagenic ETEC strain (E1392/75/2A) had previously been isolated and tested in phase I studies as a potential vaccine. This is a CS1, CS3 expressing CFAII strain of th O6:H16 serotype. While providing significant protection against challenge in volunteers, it still caused low grade diarrhea in 15% of recipients. To further attenuate this strain, two deletion mutations were introduced into the chromosome of E1392/2A. The first strain (PTL-ETEC-002) is deleted in *aroC* and *ompR* genes and the second strain (PTL-ETEC-003) is deleted in *aroC, ompC* and *ompF*. *AroC* is the gene encoding chorismate synthase in the aromatic amino acid biosynthetic pathway. *OmpR* encodes a regulatory protein

which controls the inverse regulation of *ompC* and *ompF*, encoding outer membrane porins expressed at high and low osmotic pressure, and certain other genes including those responsible for the expression of Vi antigen in *S.typhi*. Phenotypically both sets of mutations are expected to reduce the ability of the organism to adapt to the conditions in the human digestive tract, attenuating its ability to colonise and cause disease.

STUDY OBJECTIVES

To recruit volunteers according to the protocol's inclusion and exclusion criteria, to admit these volunteers to the General Clinical Research Center (GCRC) and give them a dose of vaccine candidate, to monitor them clinically and manage any symptoms which might occur.

To monitor the fecal excretion of the vaccine candidate strains.

To measure the serological response as determined by the antibody titers in the serum and the supernate of lymphocytes cultured *in vitro*.

INVESTIGATIONAL PLAN

6.1 Overall Study Design and Plan-Description

The trial was designed as a 30 subject (15 for each vaccine), single-center, open-label, inpatient, safety and immunogenicity study to evaluate two new attenuated strains of enterotoxigenic *E. coli* vaccin (PTL-ETEC-002 and PTL-ETEC-003) given in a dose-escalation design with escalation to the highe dose level dependent on lack of clinically significant effects at the lower dose level, the planned escalation was from 5 x 10^7 bacteria to 5 x 10^9 bacteria to 5 x 10^{10} bacteria. The protocol and the cas report form are attached in **Appendices 12.1** and **12.3** respectively.

6.2 Discussion of Study Design

Since the primary objective of the trial was to evaluate the safety of two new attenuated vaccin strains, an open-label planned dose escalation design was deemed appropriate.

Fecal excretion and immunological parameters would be evaluated by dose group. Vaccine wa administered orally as a single dose on Day 0 to eligible inpatient volunteers. Both vaccine strain were given in a dose-escalation design with escalation to the higher dose level dependent on lack o clinically significant effects at the lower dose level, the planned escalation was from 5 x 10^7 bacteria to 5 x 10^9 bacteria to 5 x 10^{10} bacteria.

6.3 Selection of Study Population

6.3.1 Inclusion Criteria

The following inclusion criteria were applied:

a. healthy, male or female inpatient volunteers, >18 or <50 years of age,
b. completed training on ETEC, diarrhea and protocol procedures,
c. demonstrate comprehension of the protocol procedures and knowledge of diarrhea, ETEC bacteria by passing a written examination, and
d. provide written informed consent.

6.3.2 Exclusion Criteria

The following exclusion criteria were applied:

a. chronic illness,
b. immunosuppressive condition,
c. positive serology for HbsAg, HCV, and/or HIV,
d. positive urine HCG within 4 days of immunization (women only),
e. antibiotics used within 7 days of vaccination,
f. significant abnormality in screening laboratory examinations (complete blood count with differential, blood chemistry, urinalysis),
g. if they travelled to a developing country within 5 years,
h. if they previously participated in an ETEC study,
i. regular use of laxatives or abnormal stool pattern, and
j. volunteers over the age of 40 with a abnormal EKG.

6.4 Removal of Subjects From Treatment or Assessment

Subjects were removed from the study if, in the opinion of the investigator, the health status of th subject warranted withdrawal (either through an adverse event or concurrent illness), there wa significant non-compliance with the protocolled assessments or visits, or consent was withdrawn.

Where possible, follow-up assessments were conducted as protocolled, to the end of the appropriat treatment period (i.e. 14 days post vaccination) in all subjects who were withdrawn.

Subjects who were withdrawn from the study were not replaced. Twenty-nine subjects (29) wer screened for this study and 27 were enrolled. Two screened subjects (#18 and #24) withdrew from th study prior to enrollment; the former for personal reasons and the latter secondary to hyperglycemia.

6.4.1. Discontinuation of Treatment in a Specific Cohort of Subjects

The open-label study allowed both vaccine strains to be given in a dose-escalation design with escalation to the higher dose level dependent on lack of clinically significant effects at th lower dose level, the planned escalation was from 5×10^7 bacteria to 5×10^9 bacteria to 5×10^1 bacteria.

6.5 Treatments

6.5.1 Treatments Administered

For the three dose groups, on Day 0 stomach contents were buffered with 120ml sodium bicarbonate solution (1.33% w/v), immediately prior to the oral administration of either 5 x 10 bacteria, 5×10^9 bacteria, or 5×10^8 bacteria suspended in 30ml of sodium bicarbonate solution

6.5.2 Identity of Investigational Product

The vials of seed lots of strains PTL-ETEC-002 and PTL-ETEC-003 (100 vials per lot of each strain) were supplied by Peptide Therapeutics, Ltd. (100, Fulbourn Rd., Cambridge CB1 9PT United Kingdom) in Xml clear, neutral type 1 glass vials sealed with grey butyl rubbe stoppers. Batch numbers [insert] were used. Certificates of analysis for both batches are given in **Appendix 12.7**.

Vaccine supplies were stored at –70°C in a temperature-monitored secure freezer on th inpatient unit of the GCRC?. The study coordinator maintained vaccine accountability documentation. Prior to, and on completion of the trial, any counts performed? If yes where.

Buffer solution comprised a 1.33 % w/v solution of sodium bicarbonate (? Laboratories) in water for injection.

6.6 Method of Assigning Subjects to Treatment Groups

Eligible subjects in the groups were sequentially assigned as they were screened to one of the two strains of bacteria.

6.7 Selection of Doses in the Study

The dose ranges of 5 x 10^7 to 5 x 10^{10} were chosen (why/rationale). In that study, 3 of 12 subject experienced diarrhea X days after vaccination with a dose of 5 x 10^{10}.

6.8 Selection and Timing of Dose for Each Subject

As describe in **Section 7.1**, subjects were sequentially assigned into one of two strains enrolled into the first dose group. Each group was assessed for clinical significant effects before proceeding to th next dose group. Each comprised the oral administration of 120ml of sodium bicarbonate solution to neutralize the gastric acid (which would otherwise diminish the potency of the vaccine), followed immediately by 30ml of vaccine bacteria suspended in buffer.

Volunteers were requested to fast for 90 minutes before and after administration of vaccine and wer observed to ensure consumption of the entire contents of each vaccine.

6.9 Blinding

This was an open-label study and therefore no blinding mechanisms required or implemented.

6.10 Prior and Concomitant Therapy

Prior ETEC vaccination at any time, or treatment with antibiotics within 7 days of vaccination wa prohibited. Anti-pyretics were not permitted during the follow-up period unless discussed beforehand with study personnel. This was to avoid masking of any vaccine-induced fever.

6.11 Treatment Compliance

All vaccinations were conducted in the inpatient unit at GCRC at the Johns Hopkins Hospital. A member of the VTU staff witnessed that the buffer and vaccine solutions were completely consumed and documented on the vaccine accountability record.

6.12 Efficacy and Safety Variables

6.12.1 Efficacy and Safety Assessments

A direct assessment of efficacy (i.e. protection against ETEC) was not made or planned in thi trial.

<u>Excretion of the vaccine strains</u>

Up to two stool specimens were collected each day after the immunization and were cultured on MacConkey agar and on MacConkey agar with streptomycin. Colonies that grew on th Mac-strep plate were presumed to be vaccine strains and five colonies were spotted onto Luri agar and onto minimal media (Davies). Control (wild type) strains of *E. coli* grow on both o these agars, but the vaccine strains do not grow on the minimal media. At least one colony o the vaccine strains was saved on nutrient agar slants.

Serology

Serum and lymphocyte specimens were obtained on day of immunization and on days 7 and10 after the immunization, an addition serum sample was collected 14 days after immunization. The serum specimens were assayed by ELISA for IgG antibodies to the CFA/II antigen using antigen provided by Peptide Therapeutics. The assay was performed by pre-coating the plat with CFA/II antigen using a concentration of 1 μg/mL in PBS. After an overnight incubation at room temperature, the plates were blocked with BSA and washed. Three-fold dilutions o the volunteers' sera were prepared starting with a dilution of 1:10 in the first cup. The plate were incubated for one hour and subsequent steps of incubation with HRP-labeled anti-human IgG and substrate. Between each step, the plates were washed with PBS-Tween 20. The plate were read in an automatic ELISA reader.

To establish the appropriate concentration of CFA antigen for the assay, a validation study wa carried out using varying dilutions of antigen, and sera from mice. Serum #1 was from mic that had been immunized with a CFA/II bearing strain. Serum #2 was from mice immunized with an isogenic strain without CFA/II expression, and serum #3 was from mice that had no been immunized. Using these reagents, the titration results were similar when th concentration of the CFA/II varied from 5 to 45 μg/mL. There was a slight drop in Absorbance values when the antigen concentration was lowered to 1 μg, and a major drop when the concentration was lowered to 0.2 μg. The titers of the two immunized mice wer higher than the serum from the non-immunized mice, but the serum from the mice immunized with CFA-negative *E. coli* was significantly higher than the non-immunized mouse serum suggesting that the CFA antigen contained some antigens from the bacteria in addition to CFA antigen. The concentration of 1 μg/mL appeared to be optimal for differentiating the two immune mice sera.

A standard serum pool was developed as a positive control. To make the standard, 0.3 mL o sera collected on day 10 from those volunteers who had received the dose of 10^9 CFU wer pooled. The test serum from each volunteer was tested on the same plate on the same day and a titration of the standard serum was included on each plate.

Safety

Safety was assessed by way of investigator assessment twice daily, vital signs were taken thre times a day and fluid intake and output were measured on Days 0 through 6 post vaccination.

All stools were examined, graded and weighed by the nurse. The first two stools each day wer to be sampled for microbiological examination and tested for occult blood. The stoo consistency was graded as 1=formed, 2=soft/mushy, 3=thick liquid, 4=opaque watery, 5=ric in water.

Diarrhea was defined as two or more loose stools (≥ grade 3 stools) in a period of 24 hour totalling 200 grams, or the occurrence of a single loose stool with a weight of 300 grams o more.

Dysentery was defined as the occurrence of diarrhea with blood in the stool as detected a grossly visible blood.

A fever was defined as the occurrence of an oral temperature >38.0°C sustained in at least two occasions four hours apart. Where oral temperatures of ≥38.0°C were recorded on two occasions four hours apart prior to Day 4, appropriate cultures were obtained and ciprofloxacin (500mg BID for X days) was prescribed. If no symptoms developed following vaccination, th volunteers were given ciproflaxin (500mg BID) for 3 days beginning on Day 4.

For the assessment of reactogenicity, all signs and symptoms of grade 1 or more wer reviewed. Signs assessed include ill appearance, rash, abdominal tenderness, liver palpable o spleen palpable. The signs were assessed as Yes=present or No=not present. Symptom assessed include feels ill, poor appetite, nausea, vomiting, abdominal gurgling, gas, abdomina cramps, diarrhea, tenesmus, chills, malaise, bedridden, headache, lightheaded, and muscl aches. The symptoms were graded as 0=none, 1=mild; elicited on questions, 2=moderate; sel reported, 3=severe; symptoms interfere with normal function.

A serious adverse event (SAE) was defined as any untoward medical occurrence that at any dose: results in death, is life-threatening, requires or prolongs hospitalization, results in persistent or significant disability/incapacity, or was a congential abnormality/birth defect. I was standard operating procedure of the GCRC and VTU to provide preliminary information on the SAE to the investigator who reported the SAE to the FDA, JCCI IRB and Peptid Therapeutics within 24 hours of the knowledge of such an event.

Additional safety assessments included the determination of the extent and duration o bacterial shedding, by the collection of stool samples on Days 1-6 after vaccination.

At the discharge visit from the inpatient facility (Day 6) subjects were asked to contact th VTU if any signs and symptoms occurred in the succeeding 8 days.

6.13 Appropriateness of Measurements

Other clinical studies with ETEC vaccines have indicated that serum and intestinal antibodies to CFA II antigens are appropriate measures of responses to vaccination.

Because of the inpatient design of this study, the collection and evaluation of the stools samples wa feasible.

For documentation of safety, daily assessments of signs and symptoms, and vital signs in the inpatien facility was a consistent method of data collection and evaluation.

6.14 Drug Concentration Measurements

The measurement of circulating drug levels for a vaccine is considered inappropriate, since antibody titres and their duration are the primary measure of vaccine efficacy, both of which are unrelated to systemic drug concentration. Consequently systemic drug levels were not measured.

6.15 Data Management and Quality Assurance

All hematology, clinical chemistry and urinalysis samples were analyzed by a quality assuranc accredited laboratory (certificate of accreditation is given in **Appendix 12.6**). No specific quality assurance systems were applied to the immunological assays, which were conducted at (insert).

Source document verification as applicable to completion of case report forms was not carried out fo this Investigator IND Study.

All clinical data i.e., all data with the exception of the bacteriological and immunological assays, wer obtained by GCRC and VTU personnel and reside on the hard copy forms. The bacteriological and immunological data reside in notebooks and Excel spreadsheets kept at the VTU.

6.16 Statistical Methods Planned in Protocol and Determination of Sample Size

6.16.1 Statistical and Analytical Plan

There was no formal statistical analysis plan for this Phase I trial.

Safety was evaluated by reviewing the completed case report forms and laboratory values fo individual subjects by treatment group.

For each immunogenicity endpoint, the null hypothesis for all immunogenicity comparison was that the immune response was the same for the two vaccine strains and across dose levels.

6.16.2 Determination of Sample Size

The number of subjects planned for the study was based on logistical considerations rather than power calculations.

6.17 Protocol Amendments

No fundamental changes were made to the planned analyses described in the protocol. No protoco amendments were made during the conduct of this inpatient protocol.

STUDY SUBJECTS

7.1 Disposition of Subjects

Twenty nine (29) subjects were screened and a total of 27 healthy adult inpatient volunteers from the Greate Baltimore area were sequentially assigned into one of two strains of bacteria into dose escalation groups 5 x 10^7 bacteria to 5 x 10^9 bacteria to 5 x 10^8 bacteria, as shown in **Table 1** and **Table 2**. Two screened subjects (#18 and #24) withdrew from the study before receiving vaccine, the former for personal reason and the latter secondary to hyperglycemia.

Table 1
Number of Subjects Planned and Analyzed

# Subjects Planned	# Subjects Screened	# Subjects Completed	# Subjects Withdrew
30	29	27	2

Table 2 Allocation of Treatment to Inpatient Volunteers			
Group	**PTL-ETEC-002**	**PTL-ETEC-003**	**Total**
5.7 X10^7	3	3	6
5.7 X10^9	5	6	11
5.7 X10^8	6	4	10

Key demographic variables of age, sex, race and treatment group are summarized in **Table 3** and listed by subject in **Appendix 12.8.** Of the 27 healthy subjects, 22 were male and 5 were female; 24 were african american, 2 were caucasian, and 1 was asian, with a age range from 18 to 50 years.

Table 3 Demographics						
Variable[1]	**Treatment Group**					
	PTL-ETEC-002			**PTL-ETEC-003**		
	5 x 10^7	**5 x 10^8**	**5 x 10^9**	**5 x 10^7**	**5 x 10^8**	**5 x 10^9**
Age (Yrs.)						
Mean	36	41	36	46	27	34
S. D.	9	8	9	2	6	8
Range	28-45	24-46	24-43	44-48	21-34	26-44
Gender						
Male	3	4	4	3	3	5
Female	0	2	1	0	1	1
Ethnicity						
Caucasian	1	0	0	0	0	1
African-American	2	6	5	3	3	5
Asian	0	0	0	0	1	0
Other	0	0	0	0	0	0

[1] Demographics determined at the time of vaccination.

7.2 Protocol deviations

No formal protocol deviations were documented. In total 27 of a possible 30 vaccinations wer administered. It was thought that these were sufficient numbers exposed to each strain to fulfill th objectives of the trial.

All volunteers attended the required number of outpatient visits as scheduled in the protocol. It is noted that there are study related procedures that were not consistently documented on the case report form and/or source documents as delineated in the protocol.

7.3 Extent of Exposure

The number of subjects who received treatment is shown in **Table 4**.

Table 4 Treatment Groups			
Group #	**Vaccine Strain**	**Dose**	**# Subjects**
1	PTL-ETEC-002	5×10^7	3
	PTL-ETEC-003	5×10^7	3
2	PTL-ETEC-002	5×10^9	6
3	PTL-ETEC-003	5×10^9	6
4	PTL-ETEC-002	$5 \times 10^{10*}$	6
5	PTL-ETEC-003	$5 \times 10^{10*}$	6

* These groups were replaced with a 5×10^8 CFU dose level as adverse events were experienced in the 5×10^9 CFU groups.

In all cases, the vaccine solution was fully consumed. There were two cases of vomiting, one being mild and within the first 24 hours of vaccination, and one moderate case >24 hours after vaccination (volume for either case was not recorded). The subject with the mild case of vomiting proceeded with eating after vomiting. It is assumed that the vaccine was completely ingested in both cases.

EFFICACY EVALUATION

8.1 Data Sets Analysed

There were no subjects for whom pre-vaccination or post-vaccination samples were missing. Al available data from the 27 subjects who received a dose of either the live vaccine strain PTL-ETEC 002 or PTL-ETEC-003 were reviewed and samples analyzed.

8.2 Serology

The titers of the IgG and IgA anti-CFA/II did not change significantly between the sample collected prior to vaccination and those collected after vaccination, although there was great variability between the titers from one volunteer to others. The titers are shown on **Table 5**.

Peripheral blood mononuclear cells (PBMLs) collected on Days 0, 7, and 10 were analyzed by antibody lymphocyte supernatant assay (ALS). Unstimulated PBMLs were cultured for 48 hours and the culture supernatant subjected to ELISA assay for CFA/II specific IgG or IgA. IgG and IgA titer by ALS increased significantly in the Day 7 sample compared to the preimmune sample collected on Day 0. By Day 10, the titers have decreased (**Table 5**).

Table 5 Immune Responses to CFA/II Antigen						
Strain	**Dose**	**Day**	**Anti CFA/II Titers; GMT (95% confidence interval)**			
			Serum IgG ELISA	**Serum IgA ELISA**	**IgG ALS**	**IgA ALS**
PTL-002	All	0	527(329-845)	256(172-380)	0.141(0.1-0.21)	0.08(0.04-0.15)
		7	531(331-852)	273(174-429)	0.285(0.16-0.52)	1.007(0.38-2.65)
		10	533(332-858)	308(189-503)	0.18(0.12-0.26)	0.1(0.04-0.23)
		14	527(326-855)	274(180-416)	Not Done	Not Done
PTL-002	5×10^7	0	538(75-3882)	336(131-861)	0.158(0.1-0.25)	0.124(0.03-0.5)
		7	524(79-3504)	329(57-1883)	0.587(0.14-2.55)	13.2(5.3-33.2)
		10	531(76-3727)	363(121-1088)	0.279(.14-.58)	0.346(0.07-1.67)
		14	512(73-3595)	366(131-1023)	Not Done	Not Done

Table 5 Immune Responses to CFA/II Antigen						
Strain	**Dose**	**Day**	**Anti CFA/II Titers; GMT (95% confidence interval)**			
			Serum IgG ELISA	**Serum IgA ELISA**	**IgG ALS**	**IgA ALS**
PTL-002	5×10^8	0	609(325-1143)	180(122-264)	0.11(0.06-0.21)	0.06(0.03-0.14)
		7	594(306-1156)	185(127-270)	0.109(0.06-0.21)	0.203(0.16-0.27)
		10	606(323-1137)	196(114-338)	0.112(0.06-0.2)	0.052(0.02-0.13)
		14	623(312-1248)	188(138-256)	Not Done	Not Done
PTL-002	5×10^9	0	437(278-689)	332(145-760)	0.175(0.08-0.36)	0.087(0.03-0.29)
		7	466(281-775)	402(170-953)	0.585(0.37-0.93)	1.464(0.48-4.43)
		10	459(271-779)	401(163-986)	0.245(0.16-0.37)	0.105(0.02-0.55)
		14	438(283-681)	360(143-907)	Not Done	Not Done
PTL-003	All	0	355(230-548)	141(83-242)	0.083(0.05-0.13)	0.102(0.05-0.02)
		7	383(256-572)	190(117-308)	0.325(0.16-0.64)	0.749(0.35-1.6)
		10	389(249-605)	201(124-326)	0.194(0.11-0.34)	0.388(0.17-0.86)
		14	354(235-533)	181(115-286)	Not Done	Not Done
PTL-003	5×10^7	0	365(84-1588)	470(320-688)	0.059(0.02-0.19)	0.235(0.07-0.77)
		7	384(91-1617)	502(320-790)	0.143(0.06-0.36)	0.342(0.14-0.83)
		10	359(83-1555)	503(325-777)	0.233(0.11-0.49)	0.471(0.15-1.45)
		14	348(82-1474)	467(255-855)	Not Done	Not Done
PTL-003	5×10^8	0	331(165-662)	128(70-234)	0.097(0.08-0.11)	0.057(0.02-0.13)
		7	366(193-696)	142(70-290)	0.148(0.04-0.58)	0.304(0.07-1.29)
		10	91(4.5-1832)	129(52-315)	0.063(0.04-0.1)	0.054(0.03-0.11)
		14	335(162-690)	133(70-250)	Not Done	Not Done
PTL-003	5×10^9	0	366(202-663)	83(40-173)	0.088(0.04-0.22)	0.1(0.03-0.31)
		7	393(235-655)	142(70-288)	0.83(0.51-1.35)	2.025(0.96-4.27)
		10	394(231-674)	159(85-297)	0.246(0.11-0.54)	0.939(0.52-1.69)
		14	371(227-605)	138(73-261)	Not Done	Not Done

.3 Efficacy/Immunogenicity Results

Two live oral vaccine strains PTL-ETEC-002 and PTL-ETEC-003 were given to inpatient volunteers at th Vaccine Testing Unit at Johns Hopkins University. They were given increasing doses of the two vaccin strains from 5×10^7 up to 5×10^9 CFU per dose using a bicarbonate buffer to neutralize stomach acid.

The vaccine strains were excreted in the stool in nearly all of the volunteers, but the excretion was mor consistent and longer when the highest dose 10^9 was administered. In this case, all volunteers continued to excrete the strain for at least 4 days and stopped immediately upon administration of ciprofloxacin (500 mg BID).

Neither IgG nor IgA serum anti-CFA/II antibody responses could be detected in any of the volunteers (**Tabl 5**). However, anti-CFA responses were seen by ALS assay of supernatant fluids collected from cultured lymphocytes (**Table 5**), this response peaked on Day 7 and then returned to baseline by Day 10. The clea response in the circulating lymphocytes demonstrates that the vaccine was expressing the CFA antigen in vivo It is not known if this immune response is protective against infection with ETEC.

SAFETY EVALUATION

9.1 Extent of Exposure

All 27 subjects who received a dose of either the live vaccine strain PTL-ETEC-002 or PTL-ETEC 003 according to protocol were included in the analyses.

9.2 Bacteriology

9.2.1 Excretion of the Vaccine Strains

From a maximum of 270 protocolled stool samples/rectal swabs (scheduled daily for the first 6 days after each vaccination), X# were collected for culture.

Up to two stool specimens were collected each day after the immunization and were cultured on MacConkey agar and on MacConkey agar with streptomycin. Colonies that grew on th Mac-strep plate were presumed to be vaccine strains and five colonies were spotted onto Luri agar and onto minimal media (Davies). Control (wild type) strains of *E. coli* grow on both o these agars, but the vaccine strains do not grow on the minimal media. At least one colony o the vaccine strains were saved on nutrient agar slants.

Tables 6A-6C described the excretion of the vaccine strains. Among those receiving a dose o 5×10^7 CFU, the vaccine was recovered from the stools of all of 6 volunteers at some time. I was recovered the same day as vaccination from two volunteers and continued to be excreted for up to four days in two volunteers. Of those who received the 5×10^8-dose level, 9 of 10 volunteers excreted the vaccine strain at some time, but one volunteer never excreted the strain Again, some volunteers continued to excrete for up to four days. Of those who received a dos of 5×10^9, all of 11 volunteers excreted the vaccine strain and all continued to excrete for fou days, compared to only 4 of 16 who received lower doses who continued to excrete for fou days ($p<0.0001$, Fisher's Exact Test). There was no difference in the frequency or duration o the excretion of the two vaccine strains when given at comparable doses.

Table 6A
Excretion of Vaccine Strain Following Oral Immunization 5×10^7 CFU

Date	Specimen #	Volunteer #1	Volunteer #2	Volunteer #3	Volunteer #4	Volunteer #5	Volunteer #6
		PTL-ETEC-002			PTL-ETEC-003		
27-Oct	1	0		0	1	0	
	2			1			
28-Oct	1	1	1		1	1	0
	2	1			1	1	
29-Oct	1	1	1	1		1	1
	2	1				1	
30-Oct	1	0	1	0	0	1	1
	2	0			0	1	
31-Oct	1	0	0	0	0	1	1
	2	0		0			
1-Nov	1	0	0	0	0	0	0
	2	0	0	0	0	0	0

ate of immunization was 27 October 1999. Antibiotic started on 31 October 1999

Table 6B

Excretion of Vaccine Strain Following Oral Immunization 5 x 10^8 CFU

Date	Spec #	Vol#19	Vol#20	Vol#21	Vol#22	Vol#23	Vol#25	Date	Spec #	Vol#26	Vol#27	Vol#28	Vol#29
		PTL-ETEC-002								PTL-ETEC-003			
20-Jan	1		1	1		0	0	23-Feb	1		1	0	
	2		1	0					2			0	
21-Jan	1	0	1	1	1	1	0	24-Feb	1	1	1	1	1
	2			1	1		0		2				
22-Jan	1		1	1	1	0	0	25-Feb	1		1	0	
	2					0	0		2			0	
23-Jan	1	1	0	0	0			26-Feb	1	1	1	0	1
	2		1		0	0	0		2			0	
24-Jan	1	1	1	0	0	0	0	27-Feb	1	0	0	0	1
	2		0		0	0			2	1	0	0	
25-Jan	1	0	0	0	0	0	0	28-Feb	1	0	0	0	0
	2	0	0	0	0	0	0		2	0	0		0
28-Jan	FU	0	0	0	0	0	0	4-Mar	FU	0	0	0	0
								9-Mar	FU	0	0	0	0

ate of immunization 20 January 2000, Antibiotic started 24 January 2000

Date of immunization 23 February 2000, Antibiotic started January 2000

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Table 6C

Excretion of Vaccine Strain Following Oral Immunization 5 x 10^9 CFU

Date	Spec #	Vol#7	Vol#9	Vol#10	Vol#11	Vol#12	Vol#8	Vol#13	Vol#14	Vol#15	Vol#16	Vol#17
		PTL-ETEC-002					PTL-ETEC-003					
17-Nov	1	0	0	0	0			0		0	0	
	2			0				0			0	
18-Nov	1	1	1	1	1	1	1	1	1	1	1	1
	2	1	1			1	1	1	1	1		1
19-Nov	1	1	1		1	1	1	1	1	1	1	1
	2		1		1	1	1	1	1			
20-Nov	1	1	1	1	1	1	1	1	1	1	1	1
	2					1	1	1				
21-Nov	1	1	1	1	1	1	1	1	1	1	1	1
	2	1	1		1	1	0	1		1		1
22-Nov	1	0	0	0	0	0	0	0	0	0	0	1
	2	0	0	0	0	0	0	0	0	0	0	1
23-Nov	1											0
	2											0
25-Nov	FU	0	0	0	0	0	0	0	0	0	0	0

ate of immunization was 17 November 1999, Antibiotic started on 21 November 1999

.3 **Reactogenicity**

9.3.1 Summary of symptoms

For the assessment of reactogenicity, all signs and symptoms of grade 1 or more were reviewed. Sign assessed include ill appearance, rash, abdominal tenderness, liver palpable or spleen palpable. Th signs were assessed as yes=present or no=not present. Symptoms assessed include feels ill, poo appetite, nausea, vomiting, abdominal gurgling, gas, abdominal cramps, diarrhea, tenesmus, chills malaise, bedridden, headache, lightheaded, and muscle aches. The symptoms were graded as 0=none 1=mild; elicited on questions, 2=moderate; self reported, 3=severe; symptoms interfere with norma function.

5 x 10^7 bacteria: Three (3) volunteers received strain PTL-ETEC-002 and 3 volunteers received strain PTL-ETEC-003. No significant adverse events were seen and the study proceeded to the next highes group.

5 x 10^9 bacteria: Five (5) volunteers received strain PTL-ETEC-002 and 6 volunteers received strain PTL-ETEC-003. Five of eleven subjects (45%) experienced adverse events in this dose group Moderate cramps 2/5 (40%), grade 3 diarrhea (545 grams), and moderate vomiting 1/5 (20%) was seen in subjects who received strain PTL-ETEC-002, and moderate cramps in 2/6 (33%), and grade 3 diarrhea (396 grams) was seen in subjects who received strain PTL-ETEC-003 at this dose. Therefor the original planned dose of 5 x 10^{10} bacteria was not administered and instead a reduced dose of the 5 x 10^8 bacteria was administered.

5 x 10^8 bacteria: Six (6) volunteers received strain PTL-ETEC-002 and 4 volunteers received strain PTL-ETEC-003. Two (2) of 6 subjects (33%) who received strain PTL-ETEC-002 experienced adverse events; one subject had moderate cramps and another subject had mild vomiting and moderat cramps.

None of the volunteers developed an elevated temperature. In neither case of diarrhea, vomiting, o cramps did the volunteers require restricting or changing activities.

The number of symptoms following immunization is provided in **Table 7**. No subject experienced th same symptom on more than one separate occasion, however a subject may have experienced mor than one symptom and these are counted. A detailed description of the clinical signs and symptom experienced by study subjects are described in **Appendix 12.10**.

Table 7
Summary of Symptoms Following Immunization

Strain	Dose	Any symptoms	Diarrhea	Cramps	Vomiting
PTL-ETEC-002	5 x 10^7	0/3	-	-	-
	5 x 10^8	3/6	0/6	2/6	1/6
	5 x 10^9	3/5	1/5	2/5	1/5
PTL-ETEC-003	5 x 10^7	0/3	-	-	-
	5 x 10^8	0/4	-	-	-
	5 x 10^9	2/6	1/6	2/6	0/6

9.3.2 Fever

A fever was defined as the occurrence of an oral temperature >38.0°C sustained in at least two occasions four hours apart. Where oral temperatures of ≥38.0°C were recorded on two occasions fou hours apart prior to Day 4, appropriate cultures were obtained and ciprofloxacin (500mg BID for X days) was prescribed. If no symptoms developed following vaccination, the volunteers were given ciproflaxin (500mg BID for 3 days beginning on Day 4.

No fevers were recorded in subjects who ingested the vaccine.

9.3.3 Diarrhea

All stools were examined, graded and weighed by the nurse. The first two stools each day were to b sampled for microbiological examination and tested for occult blood. The stool consistency wa graded as 1=formed, 2=soft/mushy, 3=thick liquid, 4=opaque watery, 5=rice in water.

Diarrhea was defined as two or more loose stools (≥ grade 3 stools) in a period of 24 hours totalling 200 grams, or the occurrence of a single loose stool with a weight of 300 grams or more.

Dysentery was defined as the occurrence of diarrhea with blood in the stool as detected as grossly visible blood.

A total of two single episodes of diarrhea were observed in two subjects. The two subjects who experienced diarrhea in the 5 x 10^9 bacteria dose group, one received PTL-ETEC-002 and one received PTL-ETEC-003 and are summarized in **Table 8**.

Table 8
Summary of Diarrhea as Defined per Protocol

Volunteer#	Vaccine Strain	Dose	Onset DateTime	Offset Date/Time	Number of loose stools
8	PTL-003	5 x 10^9	11/22/98 07:00	11/22/98 23:00	1
10	PTL-002	5 x 10^9	11/18/98 15:00	11/18/98 23:00	1

Diarrhea was not associated with a positive stool culture, not raising questions about its association with vaccination.

9.3.4 Vomiting

A total of two single episodes of vomiting were recorded. Two subjects experienced vomiting, one in the 5 x 10^8 bacteria dose group and one in the 5 x 10^9 bacteria dose group, both received PTL-ETEC 002 and are summarized in **Table 9**. There was no apparent pattern with respect to timing of vomiting and the administration of vaccine. On the basis of these data it cannot be concluded that there is causal relationship between the ingestion of the vaccine and the occurrence of vomiting.

Table 9 Summary of PTL-ETEC-002 Vomiting				
Volunteer#	**Dose**	**Onset Day**	**Duration (days)**	**Number of episodes**
10	5×10^9	11/17/98	1	1
21	5×10^8	1/20/99	1	1

9.3.5 Adverse Events

There was no documentation specifically identified as "adverse events". It was determined tha adverse events were a subset of the symptoms reported and recorded in the medical record or on th study forms. No formal analysis was undertaken on these data.

Volunteer #10 who experienced moderate diarrhea and moderate vomiting also reported symptoms o feeling ill and a poor appetite at the same time.

9.3.6 Deaths and Other Serious Adverse Events

There were no deaths or serious adverse events reported during the course of this protocol.

9.3.7 Clinical Laboratory Evaluation

There were no clinical laboratory data evaluated for this protocol.

9.3.8 Safety Conclusions

Two live oral vaccine strains PTL-ETEC-002 and 003 were given to inpatient volunteers at th Vaccine Testing Unit at Johns Hopkins University. They were given increasing doses of the two vaccine strains from 5×10^7 up to 5×10^9 CFU per dose using a bicarbonate buffer to neutraliz stomach acid. In general the vaccines were well tolerated; however, some moderate gastrointestina symptoms were seen in those who received doses of 5×10^9 including cramps, diarrhea and one cas of vomiting. Some mild to moderate gastrointestinal symptoms were seen in those who received PTL ETEC-002 dose 5×10^8 including cramps and one case of mild vomiting. None of the symptom restricted activities nor were they considered serious. The symptoms were not seen in the volunteer who received either 5×10^7 or 5×10^8 CFU doses.

0 DISCUSSION AND OVERALL CONCLUSIONS

The vaccine strains were associated with mild and moderate symptoms by protocol and/or case report form definition. Neither IgG nor IgA serum anti-CFA I antibody responses were detected in any of the volunteers Anti-CFA responses were seen in by the ALS assay which measures antibodies secreted from cultured peripheral blood lymphocytes, this response peaked on Day 7-post vaccination and returned to near baselin by Day 10-post vaccination.

These data suggest that both vaccine strains are well tolerated and the data form a basis for further evaluation of PTL-ETEC 002 and PTL-ETEC 003 as an outpatient study. The outpatient study should include an assessment of duration of excretion and a placebo control group to better assess the relation of symptoms with the vaccines. The lymphocyte antibody response should also be continued in the outpatient study, as i appeared toa more sensitive immune response indicator than serm antibodies.

1 REFERENCES

2 APPENDICES

Protocol and Protocol Amendments

Sample Informed Consent

12.1 Sample Case Report Form

12.2 IRB Approval(s) and Correspondence

12.3 Key Study Personnel Curriculum Vitae

12.4 Documentation of Laboratory Certification(s) and/or Quality Standards

12.5 Certificates of Analysis of Investigational Product Need

12.6 Vaccine Preparation and Dispensing Instructions Need

12.7 Demographic Data

12.8 Summary of Signs and Symptoms

12.9 Laboratory Results by Subject Need?

12.10 Vital Signs Need?

APPENDIX 12.1

PROTOCOL AND PROTOCOL AMENDMENTS

APPENDIX 12.2

SAMPLE INFORMED CONSENT

APPENDIX 12.3

SAMPLE CASE REPORT FORMS

APPENDIX 12.4

IRB APPERVAL(S) AND CORRESPONDENCE

APPENDIX 12.5

KEY STUDY PERSONNEL CURRICULUM VITAE

APPENDIX 12.6

DOCUMENTATION OF LABORATORY CERTIFICATION(S) AND/OR QUALITY STANDARDS

APPENDIX 12.7

CERTIFICATES OF ANALYSIS OF INVESTIGATIONAL PRODUCT

APPENDIX 12.8

VACCINE PREPARATION AND DISPENSING INSTRUCTIONS

APPENDIX 12.9

DEMOGRAPHIC DATA

VTU 983 DEMOGRAPHICS INPATIENT STUDY						
Subject#	**Subject ID**	**Group**	**Dose**	**Sex**	**Date of Birth**	**Race**
1	HEN	PTL-ETEC-002	5.7 X10^7	M	08-15-64	C
2	V-G	PTL-ETEC-002	5.7 X10^7	M	12-26-69	AA
3	H-L	PTL-ETEC-002	5.7 X10^7	M	01-06-53	AA
4	M-B	PTL-ETEC-003	5.7 X10^7	M	12-05-53	AA
5	CKC	PTL-ETEC-003	5.7 X10^7	M	05-14-50	AA
6	HCT	PTL-ETEC-003	5.7 X10^7	M	04-26-53	AA
7	JNM	PTL-ETEC-002	5.7 X10^9	M	07-07-69	AA
9	H-P	PTL-ETEC-002	5.7 X10^9	M	07-06-55	AA
10	SEB	PTL-ETEC-002	5.7 X10^9	F	12-10-75	AA
11	DNH	PTL-ETEC-002	5.7 X10^9	M	11-29-57	AA
12	MJT	PTL-ETEC-002	5.7 X10^9	M	06-02-55	AA
8	F-M	PTL-ETEC-003	5.7 X10^9	M	10-25-54	AA
13	A-H	PTL-ETEC-003	5.7 X10^9	M	11-30-55	AA
14	JTJ	PTL-ETEC-003	5.7 X10^9	M	10-25-71	AA
15	TAB	PTL-ETEC-003	5.7 X10^9	M	04-02-71	AA
16	MAS	PTL-ETEC-003	5.7 X10^9	M	11-18-71	AA
17	CMA	PTL-ETEC-003	5.7 X10^9	F	10-10-60	C
19	LAH	PTL-ETEC-002	5.7 X10^8	F	12-30-57	AA
20	DAW	PTL-ETEC-002	5.7 X10^8	M	07-20-54	AA
21	ZCS	PTL-ETEC-002	5.7 X10^8	F	07-22-74	AA
22	JWT	PTL-ETEC-002	5.7 X10^8	M	04-17-52	AA
23	H-I	PTL-ETEC-002	5.7 X10^8	M	03-17-53	AA
25	WDT	PTL-ETEC-002	5.7 X10^8	M	06-24-54	AA
26	BAT	PTL-ETEC-003	5.7 X10^8	F	05-23-70	AA
27	WAM	PTL-ETEC-003	5.7 X10^8	M	04-19-77	AA
28	TAW	PTL-ETEC-003	5.7 X10^8	M	04-11-64	AA
29	D-V	PTL-ETEC-003	5.7 X10^8	M	03-0375	ASIAN

A=African American; C=Caucasian

APPENDIX 12.10

SUMMARY OF SIGNS AND SYMPTOMS

Summary of Signs and Symptoms Post Immunization

Vol #	Initials	Vaccine	Dose	Date	Any Sx?	Diarrhea (y/n)	Diarrheal volume	Vomit (y/n)	Cramps (y/n)	Other Symptoms	Severity
1	HEN	PTL-ETEC-002	5.7x10(7)	27-Oct	N	N	NA	N	N	-	-
2	V-G	PTL-ETEC-002	5.7x10(7)	27-Oct	N	N	NA	N	N	-	-
3	H-L	PTL-ETEC-002	5.7x10(7)	27-Oct	N	N	NA	N	N	-	-
4	M-B	PTL-ETEC-003	6.8x10(7)	27-Oct	N	N	NA	N	N	-	-
5	CKC	PTL-ETEC-003	6.8x10(7)	27-Oct	N	N	NA	N	N	-	-
6	HCT	PTL-ETEC-003	6.8x10(7)	27-Oct	N	N	NA	N	N	-	-
7	JNM	PTL-ETEC-002	4.9x10(9)	17-Nov	Y	N	NA	N	Y	Gas	moderate
9	H-P	PTL-ETEC-002	4.9x10(9)	17-Nov	Y	N	NA	N	Y	Gas	moderate
10	SEB	PTL-ETEC-002	4.9x10(9)	17-Nov	Y	Y	545	Y	N	Feels ill, Poor appetite	moderate/ moderate
11	DNH	PTL-ETEC-002	4.9x10(9)	17-Nov	N	N	NA	N	N	-	-
12	MJT	PTL-ETEC-002	4.9x10(9)	17-Nov	N	N	NA	N	N	-	-
8	F-M	PTL-ETEC-003	4.7x10(9)	17-Nov	Y	Y	396	N	Y	Gas	moderate
13	A-H	PTL-ETEC-003	4.7x10(9)	17-Nov	N	N	NA	N	N	-	-
14	JFJ	PTL-ETEC-003	4.7x10(9)	17-Nov	N	N	NA	N	N	-	-
15	TAB	PTL-ETEC-003	4.7x10(9)	17-Nov	N	N	NA	N	N	-	-
16	M-S	PTL-ETEC-003	4.7x10(9)	17-Nov	N	N	NA	N	N	-	-
17	CMA	PTL-ETEC-003	4.7x10(9)	17-Nov	Y	N	NA	N	Y	Heartburn	moderate/ moderate
18	DROPPED										
19	LAH	PTL-ETEC-002	1.4X10(8)	20-Jan	N	N	NA	N	Y	???	???
20	DAW	PTL-ETEC-002	1.4X10(8)	20-Jan	N	N	NA	N	N	-	-
21	ZCS	PTL-ETEC-002	1.4X10(8)	20-Jan	N	N	NA	Y	Y	-	mild/ moderate
22	JWT	PTL-ETEC-002	1.4X10(8)	20-Jan	N	N	NA	N	N	-	-
23	H-I	PTL-ETEC-002	1.4X10(8)	20-Jan	N	N	NA	N	N	-	-
24	EXCLUDED*										
25	WDT	PTL-ETEC-002	1.4X10(8)	20-Jan	N	N	NA	N	N	-	-
26	BAT	PTL-ETEC-003	3.7x10(8)	23-Feb	N	N	NA	N	N	-	-
27	WAM	PTL-ETEC-003	3.7x10(8)	23-Feb	N	N	NA	N	N	-	-
28	TAW	PTL-ETEC-003	3.7x10(8)	23-Feb	N	N	NA	N	N	-	-
29	D-V	PTL-ETEC-003	3.7x10(8)	23-Feb	N	N	NA	N	N	-	-

Subject planned for inclusion in this study but was not enrolled.

APPENDIX 12.11

LABORATORY RESULTS BY SUBJECT

APPENDIX 12.12

VITAL SIGNS